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# LIPID COMPOSITION OF SARCOLEMMAL MEMBRANES OF STRIATED MUSCLE IN RELATION TO STRESS-SUSCEPTIBILITY IN BELGIAN LANDRACE PIGS.

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In recent years, a bulk of literature has been published on PSS and the related MH syndrome which develops after administration of volatile anaesthetics of which halothane is the most common. It is now generally accepted that a rise of myoplasmic $Ca^{++}$ underlies muscular rigidity during MH. The central role played by external $Ca^{++}$ in the production of "abnormal" contractures caused in vitro in MH muscle by halothane and caffeine has been described previously (1). Caffeine is known to induce contractures on isolated striated muscle strips, either by its action on sarcoplasmic reticulum or by increasing the permeability of $Ca^{++}$ through the sarcolemma.

In the experiments described here, the isolated sarcolemmal membrane fractions were tested for their ouabaine sensitive Na-K activated ATP-ase and for their lipid composition.

## METHODS

Stress-susceptible (S) Belgian Landrace (BL) and stress-resistant (R) Large White (LW) pigs weighing 40-60 kg were used in all experiments. Muscle biopsies were taken from the m. semimembranosus and m. gracilis of pigs anaesthetised with I.V. thiopentone sodium as the only anaesthetic agent. Sarcolemmal membranes were isolated after homogenisation and digestion in 1 M NaI (2). Na-K activated ATP-ase in the presence and absence of 1 mM ouabaine was measured at 37°C in an ATP regenerating system (2). Phospholipids were determined quantitatively after hydrolysis by phospholipase D, liberating free choline which was estimated colorimetrically (Wako-Japan). Cholesterol was determined after hydrolysis with cholesterolhydrase (Wako-Japan). Glycerol was assayed enzymatically after saponification of the triglycerides with ethanolic KOH (Boehringer Mannheim Diagnostica).
The relative composition of the phosphatides was determined after precipitation in ice-cold acetone, dissolved in chloroform and separated by TLC. The fractions were viewed under UV light after spraying with an aqueous solution of 0,01 % 1-anilino-8-naphtalene sulfonate and estimated after scanning densitometry.
Total lipids (To) were extracted from the sarcolemmal membranes of m. semimembranosus and m. gracilis of 4 R and 4 S pigs with chloroform-methanol (2:1). The total fatty acid composition (TTo) and the composition of fatty acids liberated after treatment of the total lipids with lipase (FTo) were determined by TLC-GC (3). From the TTo and the FTo the proportion of fatty acids liberated by lipase ($P_L To$) was calculated as

$$P_L To = \frac{FTo}{TTo} \cdot \frac{2}{3} \cdot 100$$

## RESULTS.

Na-K ATP-ase of sarcolemmal membrane fractions isolated from m. gracilis and m. semimembranosus, and its inhibition by ouabaine, is very similar in resistant LW and sensitive BL.
No significant difference was observed, neither between the total activities nor between the ouabaine inhibited activities. A relatively important variation is common for all isolates and is probably due to the presence of foreign proteins in these relative impure preparations.
The relative low inhibition by ouabaine - a value of at least 75 % was to be expected - is probably due to the same causes.
The quantitative analysis of lipids in the isolated sarcolemmal membranes was performed on 12 R and 12 S pigs. No differences could be found neither for phospholipids nor for cholesterol or glycerol in both m. gracilis and m. semimembranosus of both groups of pigs.
The phospholipids of the same isolates consisted mainly of phosphatidylcholine, less phosphatidylethanolamine and phosphatidylserine whilst only traces of sphingomyeline and lysophosphorylcholine were found. No differences in phospholipid composition of sarcolemmal membranes in both muscles of S and R pigs could be detected.
The values of TTo, FTo and $P_L To$ for the main fatty acids (C16:0, C18:0, C18:1 and C18:2) of the S group were compared with those of the R group. (Wilcoxon's test). For the TTo values no difference was observed between the two groups. However, highly significant difference ($p < 0.01$) was found between the $P_L To$ values of the S and R pigs. In comparison with the R pigs more unsaturated fatty acids (C18:1 and C18:2) and less saturated fatty acids (C16:0 and C18:0) were liberated from the S fats by lipase treatment.

## DISCUSSION

Little is known about the binding of $Ca^{++}$ to anionic sites located in or on the cellular membranes. If anionic sites are present on the outer surface of the sarcolemma which may be neutralised by either $Na^+$ or $Ca^{++}$, occupation by $Ca^{++}$ should initiate its inward movement. However, measurements of the Na-K ATP-ase activity and its inhibition by ouabaine could not reveal any differences between the R and S pigs.
Variations between membrane structures will be determined e.a. by the kind of lipids, polar groups, interaction between different fatty acid chains, the degree of unsaturation and the quantitative relation of lipids to protein. We were not able to show any difference between the phospholipid, the cholesterol and the glycerol composition (expressed as mg/mg of protein) between muscles of MH susceptible and MH resistant pigs. However, highly significant differences were observed in the release of fatty acids by lipase on the total lipids, isolated from sarcolemmal membranes : more unsaturated fatty acids were liberated by lipase from the S pig in comparison with the R pig membranes.
It has been suggested previously that mitochondria of S pigs should contain more saturated fatty acids than those of R pigs (4). Our findings indicate a difference in the positional incorporation of the fatty acids in one of the lipid fractions of sarcolemmal membranes although no differences were observed in the total fatty acid composition.

## REFERENCES.

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